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Sublimation extraction coupled with gas chromatography-mass spectrometry: A new technique for future in situ analyses of purines and pyrimidines on Mars

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Abstract

We have developed a sublimation technique coupled with chemical derivatization and gas chromatography mass spectrometry (GC-MS) to detect nucleobases and other volatile organic compounds derived from bacteria in Mars analog materials. To demonstrate this technique, a sample of serpentine inoculated with *Escherichia coli* (*E. coli*) cells was heated to 500 °C for several seconds under Martian ambient pressure. The sublimate was collected on a cold finger, then derivatized and analyzed by GC-MS. We found that adenine, cytosine, thymine and uracil were the most abundant molecules detected in the sublimed *E. coli* extract by GC-MS. In addition, nucleobases were also detected in sublimed extracts of a deep-sea sediment sample, seawater, and soil collected from the Atacama Desert in Chile after heating the samples under the same conditions. Our results indicate that nucleobases can be easily isolated directly from natural samples using sublimation and then detected by GC-MS after chemical derivatization. The sublimation-based extraction technique is one approach that should be considered for use by future in situ instruments designed to detect organic compounds relevant to life in the Martian regolith.

Keywords: Gas chromatography-mass spectrometry (GC-MS); Mars; Nucleobases; Sublimation; Bacteria; Atacama

1. Introduction

This research is part of a continued effort to develop extraction and analytical techniques for future in situ chemical analyses of the Martian regolith. The 2009 NASA Mars Science Laboratory (MSL) rover will carry a suite of instruments designed to assess the past or present habitability of Mars. One objective of MSL will be to search for organic compounds, especially those that are relevant to terrestrial life, in rock and soil samples on the surface of Mars. The sample analysis at Mars (SAM) instrument suite on MSL will include a pyrolysis-gas chromatograph-mass spectrometer (pyr-GC-MS) to detect a wide range of organic molecules extracted from Martian surface samples.

Purines and pyrimidines, such as the nucleobases adenine, guanine, cytosine, thymine, and uracil (A, G, C, T, and U), play an important role in terrestrial biochemistry as components of DNA and RNA, molecules that are responsible for the storage, transcription, and translation of genetic information in all terrestrial life. Moreover, the detection of purines and pyrimidines in CM-type carbonaceous meteorites, such as Murchison (Van der Velden and Schwartz, 1977; Stoks and Schwartz, 1981), suggests that exogenous delivery could have been an important source of these prebiotic organic compounds on Mars. Therefore, purines and pyrimidines should be included among the targeted classes of key organic compounds in

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the search for evidence of prebiotic chemistry as well as past or present life on Mars.

In 1976, the *Viking I* and *II* spacecraft successfully landed on the surface of Mars. One of the primary objectives of the mission was to determine whether organic compounds, possibly of biological origin, were present in the upper surface layer of Mars. The *Viking* pyr-GC-MS instruments found no evidence for any organic compounds of Martian origin above a few parts per billion in the upper 10 cm of surface fines (Biemann et al., 1977). It was then argued that the presence of a powerful oxidant in the Martian regolith might have destroyed organic molecules in the samples analyzed by *Viking* (Klein, 1979). It is also possible, however, that some organic compounds may have been present below the detection limit of the GC-MS instruments. In particular, the *Viking* GC-MS instruments were not optimized for the detection of several classes of organic compounds relevant to life such as amino acids, nucleobases and carboxylic acids (e.g. Benner et al., 2000). These compounds could not have been identified by *Viking*, since they are best detected by higher-temperature GC-MS techniques or after chemical derivatization to produce molecular species that are sufficiently volatile to elute through a GC column (Mahaffy et al., 2004).

It should be pointed out that pyr-GC-MS techniques similar to that employed by *Viking* have been used to identify other classes of organic molecules derived from bacterial cells including aliphatic and aromatic hydrocarbons, amides and nitriles (Simmonds, 1970; Eudy et al., 1985; Smith et al., 1987). Based on a previous pyrolysis experiment with whole bacteria (Simmonds, 1970), it was estimated that there would have to be at least 10^5 microorganisms in a 250 mg sample analyzed by *Viking* (to correspond to ~5 parts per million in weight) in order for the GC-MS instrument to detect their pyrolysis degradation products (Anderson et al., 1972).

Amino acids and nucleic acids are the most abundant organic constituents of a "typical" bacterial cell such as *Escherichia coli* (*E. coli*), comprising roughly 57% and 24% of the total dry cell weight, respectively (Neidhardt et al., 1990). Experiments designed to simulate the pyrolytic process used by the *Viking* GC-MS, have demonstrated that most of the protein-bound amino acids (~98%) originally present in *E. coli* cells will not undergo sublimation and are destroyed during heating at 500 °C under Martian ambient pressure (Glavin et al., 2001). In contrast, nucleobases derived from nucleic acids are much more resistant to thermal degradation than protein bound amino acids and will sublime directly from native *E. coli* DNA and RNA with minimal decomposition when the cells are heated to 500 °C (Glavin et al., 2002; Glavin et al., 2004). However, if nucleobases were released from the Martian fines during the *Viking* pyrolysis procedure, they would not have been identified by the GC-MS instrument, since these compounds require chemical derivatization prior to analysis.

In this paper, we describe a sublimation-based extraction and chemical derivatization technique that could be used to isolate nucleobases directly from the Martian regolith for analysis by GC-MS. Preliminary results using this extraction technique on a variety of Martian analog materials will be presented. A simplified version of this extraction procedure that consists of a single "one-pot" chemical derivatization step has been developed for the SAM GC-MS instrument on the MSL mission. In addition, sublimation extraction will be implemented in the Mars organic detector (MOD) component of the Mars Astrobiology Probe (MAP) instrument, selected for the European Space Agency ExoMars mission (Kminek et al., 2000).

2. Experimental

2.1. Chemicals and reagents

Individual purine and pyrimidine standards (>99% purity) including adenine (A), cytosine (C), thymine (T), guanine (G), uracil (U), hypoxanthine (HX), and xanthine (X) were purchased from Sigma-Aldrich. A concentrated stock solution ($\sim 3 \times 10^4$ M) was prepared by mixing each base in 0.1 M NaOH. The derivatization reagent *N,N-tert*-butyl-dimethylsilyl-trifluoroacetamide (MTBSTFA) and pyrene standard (100 ng/μl in cyclohexane) were from Sigma-Aldrich and dimethylformamide (DMF) was from Pierce.

2.2. Sample preparation

2.2.1. E. coli cells

A crushed serpentine (hydrated magnesium silicate) sample that had been heated at 500 °C for 3 h to remove any organic material was inoculated with *E. coli* bacteria using the following procedure. *E. coli* cells (strain MG1655) were grown in glass tubes with 0.5 g of serpentine sample by shaking at 250 rpm overnight in 10 ml Luria–Bertani (LB) medium at 37 °C in a water bath. After overnight growth, a 0.5 ml aliquot of the medium was transferred into a quartz cuvette, and the optical density at 460 nm (OD_{460}) then measured using a HP 8452A diode array spectrophotometer to determine the total cell concentration in the sample.

The remaining LB growth medium was centrifuged for 10 min at 5000 rpm to pellet the cells. The medium was decanted from the tube and the bacteria/serpentine sample rinsed with 1.5 ml of potassium phosphate-buffered saline (KPBS), centrifuged at 6000 rpm for 2 min, and the supernatant removed. This KPBS washing procedure was repeated three times to remove the remaining LB medium from the sample. The inoculated serpentine sample was homogenized by mixing, vacuum-dried, weighed and then stored at 4 °C. A crushed serpentine control blank that had not been inoculated with *E. coli* cells was also carried through the LB medium treatment, KPBS washing, and mixing procedure described above.

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2.2.2. Natural samples

Several natural samples were selected as Mars analog test materials for this study. They include a seafloor sediment core from the Nile Delta, unfiltered surface seawater from La Jolla Shores in San Diego, CA, a sample of the CM2-type carbonaceous meteorite Murchison provided by the Smithsonian Natural Museum of Natural History (USNM, 6650,2), and a surface soil sample (1 cm deep) collected from the Yungai region (24°S, 70°W) of the Atacama Desert in Chile. The Atacama Desert soils have been described as "Mars-like" given the presence of oxidizing materials, trace quantities of organic compounds, and extremely low levels of culturable bacteria (Navarro-González et al., 2003). The solid samples were crushed using a mortar and pestle.

2.3. Sublimation extraction procedure

A portion of the inoculated serpentine sample was weighed (0.2 g), transferred to a quartz glass sublimation apparatus (SA, see Fig. 1), dried under vacuum, sealed at Martian ambient pressure (~4–6 torr air), and then heated at 500 °C for 30 s in a tube furnace. The interior temperature of the quartz tube where the sample was located inside was monitored during the sublimation using an Omega DP25-TC digital display meter and type K thermocouple. The heating cycle used was similar to the *Viking* GC-MS pyrolysis procedure used to extract organic compounds from Martian surface samples (Biemann et al., 1977).

A cold finger attached to the sublimation tube was kept at −195 °C with liquid nitrogen throughout the experiment. The temperature gradient inside the SA (500 °C to cold finger temperature of −195 °C) during the heating experiment facilitated the vaporization of nucleobases from the sample at the bottom of the quartz tube and their condensation on the end of the cold finger. After heating, the residue on the end of the cold finger was rinsed off in 1 ml double distilled water and the extract analyzed for nucleobases by GC-MS. Samples of the Nile delta sediment (1 g), Atacama soil (1 g), seawater (20 ml), and Murchison meteorite (0.2 g) were carried through the identical heating procedure described above and analyzed by high-performance liquid chromatography (HPLC) with UV absorption detection as described elsewhere (Glavin et al., 2004).

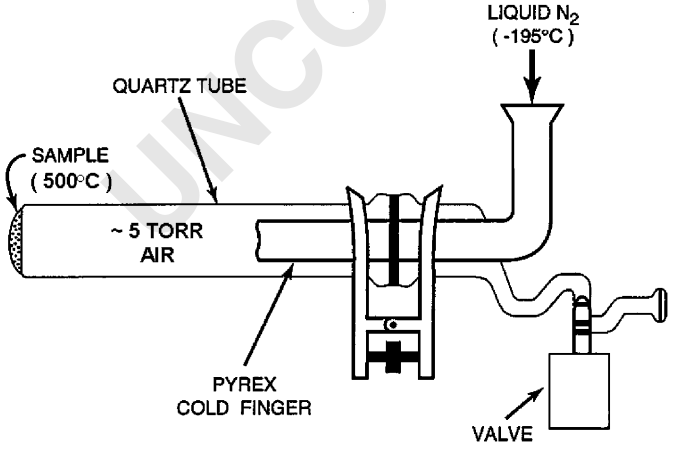

Fig. 1. Diagram of the custom-made sublimation apparatus (SA) used in the heating experiments.

2.4. Chemical derivatization and GC-MS analysis

The analysis of nucleobases by GC-MS requires a chemical derivatization step, since these compounds must be transformed into volatile derivatives that are stable in a GC column. One such reaction shown below (Fig. 2) uses MTBSTFA as the chemical derivatization agent. The MTBSTFA silylation reaction has previously been optimized for amino acids and has an advantage over other derivatization reagents since it is a single-step reaction, is less sensitive to hydrolysis, and does not require separation of the derivatives prior to GC analysis (MacKenzie et al., 1987; Rodier et al., 2001). In addition, MTBSTFA is sensitive to all compounds with acidic hydrogen atoms including primary and secondary amines, amino acids, alcohols, and carboxylic acids.

In order to determine the response linearity and detection limit for nucleobase derivatives using GC-MS, diluted aliquots of the nucleobase standard stock solution (~10^{-3} M) were evaporated inside a 1 ml glass vial under a stream of dry nitrogen. Then 30 µl MTBSTFA and 10 µl DMF were added and the vial heated at 75 °C for 2 h. The temperature and duration of heating used in the derivatization experiments were optimized to achieve maximum yields. After the sample cooled to room temperature, 5 µl of pyrene (100 ng/µl) was added as an internal standard since pyrene does not react with MTBSTFA. Finally, 1 µl of each derivatized standard mixture was injected directly into the GC-MS operated in split mode (1:15). The amount of each nucleobase injected ranged from ~3 to 300 pmol and that of pyrene (I.S.) was 55 pmol. Separation of the derivatized compounds was achieved by gas chromatography using a Restek capillary column (RTX-5MS, 15 m × 0.25 mm, 5% diphenyl, 95% dimethylpolysiloxane)

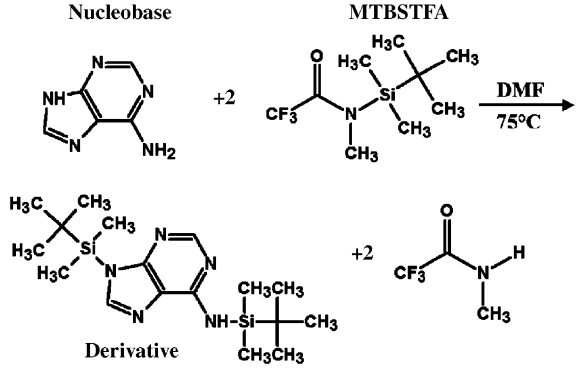

Fig. 2. Scheme of the MTBSTFA derivatization reaction that transforms non-volatile compounds such as the nucleobase adenine into a volatile derivative that can be analyzed by GC-MS.

with the He flow held constant at 1.3 ml/min. The operating conditions were as follows: initial temperature, 50 °C; ramp rate, 20 °C/min; 100 °C; ramp rate, 5 °C/min; final temperature, 250 °C. The gas chromatograph was connected to a Thermo Finnigan Trace DSQ mass spectrometer operated in quadrupole detection mode at 70 eV with the interface temperature at 280 °C. Full mass scans (50–650 amu) were collected during each run at a rate of 0.93 scans per second. For each nucleobase standard, the peak area ratio of the nucleobase to pyrene was measured and plotted against the amount of nucleobase injected. This should result in a straight line if no loss of the derivatives occurs during GC analysis and the concentration of the sample is within the range of linearity.

For the sublimed *E. coli* sample, the water extract was dispensed into a 1 ml glass vial, evaporated at 35 °C under a stream of dry nitrogen, and then derivatized as discussed above. The nucleobase derivatives were then identified by GC retention time and their unique mass fragmentation pattern. The absolute abundance of each compound in the sample was then determined from the nucleobase/pyrene peak area ratio and the corresponding calibration data of the standards.

3. Results and discussion

3.1. Standard calibration experiments

Fig. 3 shows the chromatogram of a mixture of derivatized nucleobases we obtained on a capillary GC column. The separation of all of the nucleobases in the standard mixture including uracil, thymine, cytosine, hypoxanthine, adenine, xanthine, and guanine was achieved in less than 30 min. The mass fragmentations of the silylated nucleobase derivatives are given in Table 1. All of the targeted compounds could be unambiguously identified from their major fragment ions and were quantified using pyrene as an internal standard.

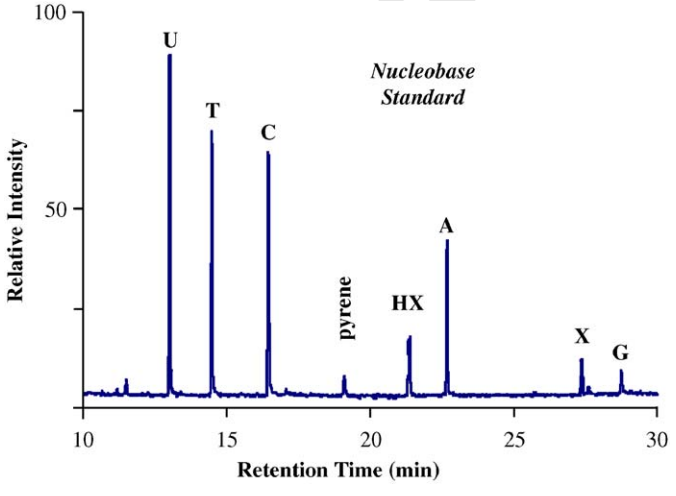

Fig. 3. GC-MS analysis of a pure mixture of nucleobases derivatized by MTBSTFA. Peak identifications: U, uracil; T, thymine; C, cytosine; HX, hypoxanthine; A, adenine; X, xanthine; and G, guanine.

The calibration graphs for all of the nucleobase derivatives tested, obtained by plotting the ratios of their peak areas at four different concentrations to that of the internal pyrene standard, showed good linearity (coefficient of regression: r^2 ~0.989–0.999) in the range of 3–300 pmol injected. Therefore, the best-fit parameters obtained from these plots were used to calculate the absolute abundances of nucleobases present in sublimed extracts. Previous experiments have shown the precision of this method to be less than 5% (Rodier et al., 2001). The GC-MS detection limits for the nucleobases tested ranged from 3 to 13 pmol (Table 1). These detection limits are ~10–100 times higher than those previously reported for amino acids using a similar analytical method (Rodier et al., 2001).

3.2. Sublimation extraction results

The chromatogram obtained after GC-MS analysis of the sublimed extract of the inoculated serpentine sample is shown in Fig. 4. Several nucleobases including uracil, cytosine, thymine, and adenine were detected in the *E. coli* cell extract. In previous sublimation experiments with λDNA, it was demonstrated that melting and fragmentation of the DNA will occur and the glycosidic bonds that attach the nucleobases to the sugar begin to break at elevated temperatures above 150 °C facilitating the sublimation of adenine, cytosine, and thymine directly from the λDNA (Glavin et al., 2002). Therefore, the nucleobases detected after sublimation of the inoculated serpentine sample were probably derived from native *E. coli* DNA and RNA. Although guanine did not sublime from the cells during the experiment, the presence of xanthine in the cold finger extract indicates that some thermal decomposition of guanine to xanthine occurred during the experiment (Fig. 4). We were unable to detect any nucleobases in the procedural blank by GC-MS (Fig. 4), which indicates that the nucleobases were derived from the *E. coli* cells and were not associated with any remnants of the growth medium.

In addition to nucleobases, several other peaks were also present in the chromatogram of the sublimed *E. coli* water-extract (Fig. 4). These organic pyrolysis products had mass fragmentation patterns similar to urea, proline, 2-piperidine, and hexadecanoic acid (a saturated fatty acid), however the yields for these compounds were not quantified. Fatty acids derived from lipids in bacterial cell walls are abundant pyrolysis products that can be identified by pyr-GC-MS (Smith and Snyder, 1992). However, since fatty acids are not very soluble in water, only trace levels were identified in our sublimed *E. coli* extract. These compounds would be best detected by GC-MS analysis of an alcohol extract (e.g. methanol) of the cold finger following sublimation heating.

With the possible exception of proline, we were unable to detect any amino acids in the sublimed extract. This result

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Table 1
Mass spectrometric fragmentation of the *tert.*-butyldimethylsilyl nucleobase derivatives obtained on a quadrupole GC-MS instrument and detection limits

Silylated nucleobase derivative	MW[a]	Fragments (relative intensities)	Detection limit (pmol)
Uracil	340	285(9) 284(26) 283(100) 282(6) 147(11) 100 (7) 99(28) 73(32) 57(22) 41(12)	3
Thymine	354	299(10) 298(24) 297(100) 147(15) 133(7) 113(21) 73(38) 59(7) 57(27) 41(14)	6
Cytosine	339	284(8) 283(27) 282(100) 212(8) 168(6) 100 (10) 98(16) 73(23) 57(20) 41(11)	4
		Hypoxanthine	364
309(8) 308(18) 307(100) 306(6) 223(5)	13	193(17) 75(5) 73(45) 57(28) 41(17)	
Adenine	363	308(8) 307(26) 306(100) 207(6) 192(25) 165(8) 73(36) 57(34) 56(6) 41(16)	5
Xanthine	494	438(25) 437(84) 265(22) 158(12) 147(12) 73(100) 57(44) 56(14) 41(28)	12
Guanine	493	437(14) 436(100) 323(11) 265(18) 264(34) 73(94) 57(38) 56(18) 45(11) 41(43)	9

[a]MW—molecular weight.

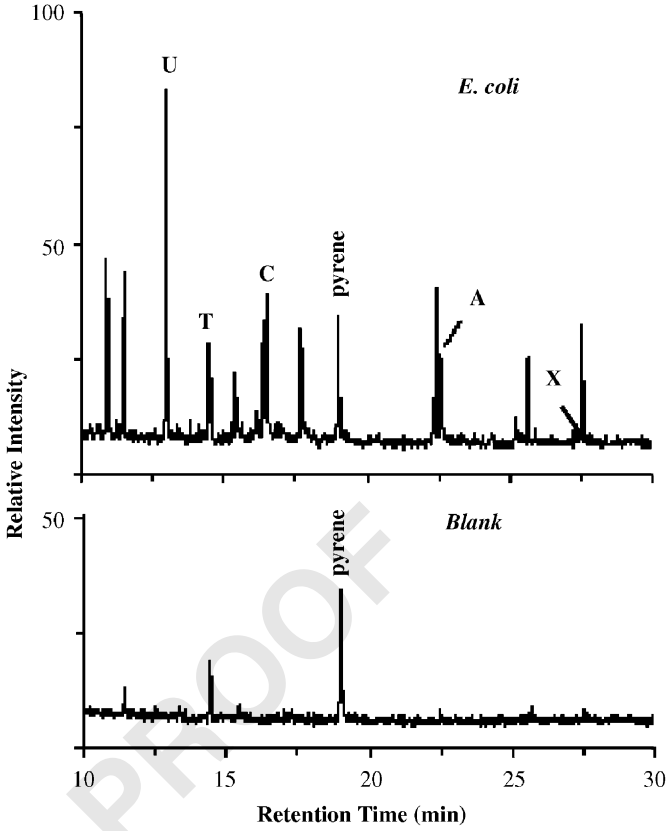

Fig. 4. GC-MS chromatograms of the sublimed extracts from a crushed serpentine sample inoculated with *E. coli* cells (top) and a procedural blank (bottom). Peaks were identified by comparison of the retention times and mass fragmentation patterns of the nucleobase standard run on the same day. Some small peaks were present in the blank with retention times similar to some of the nucleobases; however these compounds were identified as polysiloxanes from the GC column based on their mass fragmentation patterns.

was not surprising since most protein bound amino acids in the *E. coli* cells would have decomposed into amines and other products during the pyrolysis procedure (Glavin et al., 2001). Methylamine and ethylamine (amino acid decomposition products derived from glycine and alanine, respectively) were not detected in the sublimed extract using GC-MS, since the volatility of these amines increased after derivatization causing co-elution from the GC column with the derivatization solvent. Both methylamine and ethylamine, however, can be identified by GC-MS without derivatization under lower-temperature column conditions (personal communication, D. Meunier, LISA, University of Paris 12). Since amino acids in terrestrial sediments readily decompose into amines after heating at 500 °C under Martian ambient pressure (Glavin and Bada, 1998), the detection of amines released from a Martian sample by pyr-GC-MS could indicate the presence of indigenous amino acids.

3.3. Bacterial cell count estimates

3.3.1. E. coli

From the optical density measurements of the *E. coli* growth medium used to inoculate the serpentine sample, a high *E. coli* cell concentration of 1.3×10^{10} cells/g of serpentine was inferred. After heating the inoculated serpentine, the total concentration of adenine released from the sample was ~120 nmol/g measured by GC-MS. This value is equivalent to an adenine sublimation yield of 9.2×10^{-18} mol/cell based on the total number of *E. coli* bacteria in the sample and was similar to a value of 9.6×10^{-18} mol adenine per cell obtained previously using HPLC and UV absorption detection (Glavin et al., 2004). Since the adenine yield from *E. coli* after sublimation was found to be linearly dependent on the total number of cells present in the sample, the sublimed adenine yield from natural samples can be used to estimate the total number of bacterial cells (or more appropriately, *E. coli* cell equivalents (ECE)) in Mars analog samples (Glavin et al., 2004).

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Table 2
Adenine sublimation yields and bacterial cell counts in Mars analog samples (Glavin et al., 2001; Glavin et al., 2004)

Natural sample	Adenine (nmol/g)	Cell count[a] (106 ECE/g)
Serpentine (*E. coli*)	125	13,000
Nile delta sediment	13	1300
Atacama soil	0.04	4
Seawater	0.005	0.5
Murchison meteorite	<0.005	

[a]Bacterial counts reported in *E. coli* cell equivalents per gram (ECE/g). For the *E. coli* sample, this value was determined from optical density measurements of the growth medium. For the other samples, cell counts were calculated by dividing the adenine sublimation yield reported in the left column by an *E. coli* adenine recovery of 9.6×10^{-18} mol/cell from the inoculated serpentine sample.

3.3.2. Natural samples

In Table 2 the adenine yield and corresponding bacterial cell count estimates are shown for several different natural samples. Using the inoculated serpentine sample as a reference, we estimated a total cell count for the Nile delta sediment of 1.3×10^9 cells/g. This value is within the range of total microbial cell counts ($0.1–5 \times 10^9$ cells/g) reported for deep-sea sediments using traditional fluorescence staining techniques (Llobet-Brossa et al., 1998; Luna et al., 2002). For the seawater, we also calculated a sublimation based-cell count similar to counts obtained by whole cell fluorescent in situ hybridization (Maruyama and Sunamura, 2000).

Our estimate for the total bacterial concentration of the Atacama Desert surface soil (4×10^6 ECE/g) based on the adenine content is at least two orders of magnitude higher than total viable counts of culturable bacteria ($<10–10^4$ colony forming units per gram (CFU/g)) previously measured in Atacama samples by serial dilution plating (Navarro-González et al., 2003). This finding may indicate that soil samples from the Atacama Desert contain mostly non-culturable bacteria that are not detected by dilution plating. It is also possible that the soil contains mostly degraded organic matter from dead cells that are accounted for in the sublimation-based cell counts, but not by other counting methods. Contrary to earlier findings (Navarro-González et al., 2003; Maier et al., 2004), our results demonstrate that even in the driest location of the Atacama Desert, significant levels of bacteria can be detected in the upper 1 cm of the soil, but that detection is highly dependant on the technique used.

Given the measured GC-MS detection limit for adenine of 5 pmol (Table 1) and adenine sublimation yield from *E. coli* of $\sim 1 \times 10^{-17}$ mol/cell (Glavin et al., 2004), we calculate that 5×10^5 ECE/g of Martian soil should be detectable using this method. Therefore, any adenine associated with either extinct or extant life in the Martian regolith, if present at concentrations similar to the Atacama soils, would be readily detected using this technique. It is important to emphasize that it is not known if microbial life existed on Mars at some time in the past or continues to exist today. Furthermore, even if Martian life does exist, they might use an entirely different set of nucleobases in their genetic material than those found in DNA and RNA in terrestrial microorganisms. Nevertheless, if any nucleic acid-like material of Martian origin is present in the regolith, the nucleobases derived from these compounds could be identified using the sublimation extraction and GC-MS technique.

3.3.3. Murchison meteorite

After sublimation of the Murchison meteorite, we were unable to identify any nucleobases, including adenine above the 5 pmol/g level (Table 2) by either HPLC or GC-MS. This result is somewhat surprising since the purines adenine, guanine, hypoxanthine, and xanthine have previously been detected in Murchison meteorite formic acid extracts (Van der Velden and Schwartz, 1974, 1977). With an adenine concentration of ~270 parts per billion (~2000 pmol adenine/g) reported for Murchison (Van der Velden and Schwartz, 1977), this nucleobase should have been easily detected above the 5 pmol level by GC-MS assuming that most of the adenine sublimed from the meteorite during the heating procedure.

It is possible that the purines in Murchison are physically and/or chemically bound to other non-volatile organic components in the meteorite, which could inhibit sublimation. The most abundant form of organic carbon in Murchison is a complex organic polymer similar to terrestrial kerogen that consists predominantly of stacked layers of aromatic hydrocarbons linked together by aliphatic carbon chains (Krishnamurthy et al., 1992; Komiya and Shimoyama, 1996). In a previous experiment, low adenine recoveries (<2%) from humic acid that had been spiked with a pure adenine standard after sublimation heating, indicated that the kerogen-like organic polymer present in Murchison could interfere with the sublimation of adenine and other purines from the meteorite (Glavin, 2001). In order to isolate adenine from the Murchison meteorite, a formic acid extraction step prior to sublimation was required (Glavin and Bada, 2004).

The Murchison sublimation heating results have interesting implications for the search for indigenous nucleobases on Mars. Based on estimates of the influx of carbonaceous interplanetary dust particles at Mars, exogenous meteorite material could account for 2–30% of the total mass of the Martian regolith (Flynn and McKay, 1990). Since nucleobases apparently do not sublime directly from the carbonaceous chondrite Murchison, the detection of nucleobases extracted from a Martian sample using sublimation could indicate that these compounds are indigenous to the planet and not of meteoritic origin. Additional sublimation experiments on other types of carbonaceous meteorites will be required to confirm this hypothesis.

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4. Conclusion

The *E. coli* sublimation results demonstrate the feasibility of using sublimation coupled with chemical derivatization and GC-MS analysis to detect nucleobases derived from bacteria in Mars soil analogs. With chemical derivatization, the detection limit for nucleobases using GC-MS is at least several orders of magnitude more sensitive than the *Viking* GC-MS instruments for these compounds. Using the sublimation-based method, bacteria counts can be estimated from the total amount of adenine released from organic matter derived from both dead and living cells. Based on our analyses of the Atacama Desert soil samples, approximately 10^5–10^6 cells/g of Martian soil should be detectable using this technique. Since nucleobases were not released from the Murchison meteorite by sublimation, the detection of these compounds on Mars may indicate an indigenous origin of these compounds. However, the extrapolation to evidence of extinct or extant life on Mars using this approach may not be warranted, since nucleobases of local abiotic origin might also behave similarly in the sublimation extraction procedure. Sublimation extraction of organic compounds coupled with GC-MS analysis would provide for enhanced science for future in situ instrumentation on Mars and elsewhere.

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